MICRO DETERMINATION OF IODINE

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IN BIOLOGICAL MATERIALS

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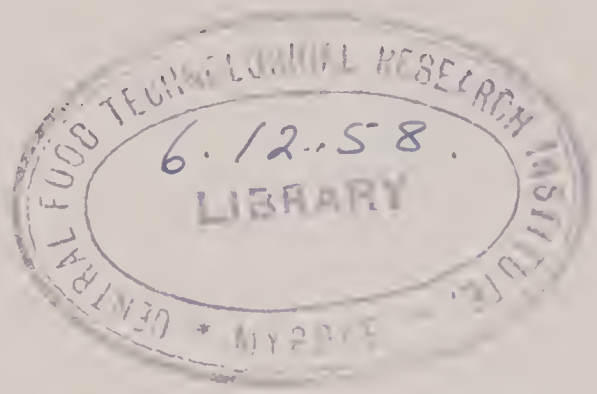

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MICRO-DETERMINATION OF IODINE IN BIOLOGICAL MATERIALS

CHEMICAL determination of relatively large amounts of iodine is a comparatively simple procedure details of which may be found in any standard textbook. When, however, the quantities to be estimated are very small, such as are normally found in body fluids, waters, soils, plant and animal tissues, a specially delicate method is required.

Early investigators of iodine biochemistry were considerably hampered by the lack of a reliable micro-technique upon which all could agree. Indeed, the results of different workers applying the same method to the same material were often at variance. In recent years this situation has vastly improved and methods are now available which in expert hands give results on which reliance can be placed.

Modern procedures for determining micro-quantities of iodine fall into two main parts: first, the destruction of organic material with which the iodine in plant and animal tissue is invariably associated, and secondly, determination by colorimetric or titrimetric means of the iodine thus isolated.

DESTRUCTION OF ORGANIC MATERIAL

Four oxidation procedures are now in use:

Chromic acid–sulphuric acid digestion. Organic material in the sample is digested in a chromic acid–sulphuric acid solution, a process which oxidizes iodine compounds to iodate. The iodate is reduced by phosphorous acid to free iodine which is isolated by distillation into a sodium hydroxide or sodium sulphite solution.

Potassium permanganate–sulphuric acid digestion. Organic matter is digested in a potassium permanganate–sulphuric acid solution. Iodine compounds are thereby converted to iodate which is then reacted with oxalic acid. Iodine thus liberated is distilled into an alkaline absorption solution.

Alkaline incineration. Organic material is incinerated at a high temperature in an alkaline medium and iodine is thereafter extracted from the ash with dilute acid.

Chloric acid digestion. Organic matter is digested in a chloric acid solution in the presence of sodium chromate. The reaction mixture is evaporated to small volume and a little water is added to dissolve the salts remaining after oxidation.

Of these four procedures, the first (chromic acid–sulphuric acid digestion) is the most widely used but has the disadvantage, shared by potassium permanganate–sulphuric acid digestion, of requiring a rather complicated distillation apparatus. No complex equipment of this kind is required for the other two oxidation methods. Alkaline incineration gives reliable results in expert hands but considerable errors are liable to occur due to loss of iodine through overheating. Chloric acid oxidation in the presence of sodium chromate, introduced by Zak and his colleagues in 1952, is the easiest procedure to carry out and is reported to give good iodine recoveries. As yet, however, it has been little used for

routine determinations and it remains to be seen whether the original claims for its reliability will be substantiated.

DETERMINATION OF IODINE

To measure the quantity of iodine thus released from organic combination one of two characteristic iodine reactions is employed:

Ceric sulphate reaction. It has long been known that iodides have a catalytic effect on the reduction of ceric sulphate by arsenious acid in sulphuric acid solution:

$$2Ce^{++++} + As^{+++} \longrightarrow 2Ce^{+++} + As^{+++++}$$

The gradual progress of the reaction can be followed by the steady disappearance of the yellow colour of the ceric ions. During work carried out in 1936, Sandell and Kolthoff (1937) found the speed of the reaction to be nearly proportional to the iodide concentration and they applied this relationship for the purpose of determining small quantities of iodides. The reaction conditions (reagent concentration, acidity, temperature) are fixed and the amounts of iodide present in test solutions are determined by measuring the time required for the complete disappearance of the yellow colour.

In 1940, Chaney adapted this procedure to the determination of iodine in blood using a preliminary chromic acid–sulphuric acid digestion of organic matter. Whereas Sandell and Kolthoff measure the time for complete reduction (decolourization) Chaney finds it more convenient to take photometric measurements after a specific time interval while the reaction is still in progress.

Chaney's method gives good results; but taking readings while reduction is still continuing can give rise to various minor errors. Accuracy may be improved by completely arresting the reaction before measuring the colour of the

test solution. Rogina and Dubravčić (1953) have described how this may be done by the addition of excess ferrous ammonium sulphate solution to the reductive system. The ferrous ions and the residual ceric ions immediately react according to the equation:

$$Ce^{++++} + Fe^{++} \longrightarrow Ce^{+++} + Fe^{+++}$$

The ferric ions produced are equivalent to the ceric ions present directly before the halting of the catalytic reaction. Chaney calculates the amount of iodine in a sample from photometric measurements of the ceric ion concentration. It follows therefore that iodine may also be estimated by measuring the ferric ion concentration. This is conveniently accomplished by adding potassium thiocyanate solution to the test solution and observing the colour intensity of the red ferric thiocyanate complex formed.

Another way of terminating the catalytic reaction has been described by Grossmann and Grossmann (1955). When brucine sulphate is added to the ceric sulphate–arsenious acid system the brucine part of the molecule removes the ceric ions by combining with them to form brucine cerate. This compound has a stable colour in solution which may be utilized for a photometric determination of iodine.

The ceric sulphate reaction has been further improved by the addition of sodium chloride to the test solution. The chloride increases sensitivity through its enhancement of the catalytic action of the iodide ion. This effect, first noted by Sandell and Kolthoff (1937), was later reinvestigated and applied to iodine analysis by Barker (1948).

Iodate–iodide reaction. Prior to the introduction of the sensitive ceric sulphate–arsenious acid reaction, the final determination of iodine was usually made by oxidizing the iodine compounds to iodate, adding a little potassium iodide to the solution, and then titrating the free iodine thus

liberated with sodium thiosulphate using starch as indicator:

$$IO'_3 + 5I' + 6H \longrightarrow 3I_2 + 3H_2O$$

As may be deduced from the equation, the amount of iodine present in the sample under examination is equivalent to one-sixth of the titratable iodine. The titration of six times the amount of " original " iodine is hailed by many as the outstanding advantage of this procedure especially since the amount of " original " iodine may be exceedingly small. Others condemn the method for this very reason; to add to the titration mixture the substance being determined (iodide to liberate " original " iodine from iodate) is, they say, a practice liable to give inaccurate quantitative results.

Nevertheless, the iodate–iodide reaction, the Dushman reaction as it is called, is still utilized in some methods, especially where the amount of iodine in the sample is relatively large as in iodized salt.

PRECAUTIONS

Because the procedures employed are so sensitive and the amounts of iodine to be determined so small (blood, milk and drinking water normally contain not more than 10 μg of iodine per 100 ml) rigorous precautions must be taken to prevent contamination from inhibitors, oxidizing-reducing agents, and outside sources of iodine. The principal points to be emphasized are: (1) Isolation of the analytical apparatus and equipment. Constant and reliable results can never be obtained if the work is carried out in proximity to other chemical processes, especially if these involve the evolution of gases, vapours or fumes. (2) The consistent use of absolutely clean glassware, and of chemical reagents of the highest possible purity. (3) Thorough destruction of the organic matter present in the sample being analysed. (4) Patience, dexterity and extreme care in manipulation.

We wish to thank The Director, Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, Weybridge, Surrey, for permission to publish this procedure. It has been in routine use for several years and is based on the published techniques of Chaney (1940), Riggs and Man (1940) and Taurog and Chaikoff (1946).

SUMMARY

Organic matter is digested with a mixture of potassium permanganate and H_2SO_4, iodine being oxidized by this process to iodate. The iodate is reduced at 140°C by oxalic acid to free iodine which is distilled into NaOH. The iodine is then determined by a colorimetric method based on the catalytic effect of iodine on the reduction of yellow ceric sulphate to colourless cerous sulphate by arsenious acid.

REAGENTS

(i) *Redistilled water* (if single distilled water is not iodine free)

Add 50 g of K_2CO_3 A.R. and a few glass beads to 1500 ml of distilled water in a 3-litre round-bottomed distilling flask mounted on a shallow sand-bath. Connect the flask to a ground-glass jointed condenser. Discard the distillate until it is neutral to methyl red indicator. Replace the alkali after 20 litres have been distilled.

(ii) *Potassium permanganate*

Dissolve 140 g of potassium permanganate A.R. in 500 ml of boiling distilled water. Allow to cool until about 10 g of coarse crystals separate out. Filter through glasswool. Stir while chilling the solution in ice-water until recrystallization is complete. Suction-filter through a Whatman No. 42 paper and wash the

crystals several times with chilled redistilled water. Transfer to an evaporating dish and dry in an oven at 100°C. (If necessary, recrystallize for the second time.) When dried, pulverize with an agate pestle and mortar until the crystals pass a 100-mesh sieve.

(iii) *Saturated oxalic acid solution*
Dissolve 500 g of oxalic acid A.R. in 400 ml of boiling distilled water. Filter through a Whatman No. 1 paper. Stir while chilling in ice-water until recrystallization is complete. Suction-filter through a Whatman No. 42 paper and wash the crystals several times with chilled redistilled water. Keep the crystals covered with saturated solution in the dark and decant for use as required.

(iv) *Ceric sulphate*
Wash 15 g of ceric sulphate (low in other rare earths) with 75 ml of boiling 95 per cent redistilled alcohol. Allow to settle and decant the supernatant liquid. Repeat the washing twice and suction-filter through a Whatman No. 42 paper. Dry at room temperature and store in a refrigerator.

(v) *Sulphuric acid* 18*N*
Add 10 ml of HCl A.R. to 2000 ml of concentrated H_2SO_4 and heat strongly for two hours to fume off the halogens. Add 170 ml of concentrated H_2SO_4 to 200 ml of redistilled water to make 340 ml of 18*N* H_2SO_4.

(vi) *Copper metal*
Wash copper foil with HCl, water and, finally, redistilled water. Dry in an oven at 100°C and cut into $\frac{1}{8}$-inch squares.

(vii) *Sodium hydroxide* 1 *per cent*
Prepare weekly from 10*N* NaOH solution in redistilled water.

(viii) *Arsenious acid* 0·15*N* *in sulphuric acid* 1·5*N*

Dissolve 3·71 g of arsenious acid A.R. in 7 ml of 10*N* NaOH solution. Add 300 ml of redistilled water and neutralize to methyl orange with 10*N* H_2SO_4 (prepared from v). Add a further 21 ml of concentrated H_2SO_4 and dilute the solution with redistilled water to 500 ml.

(ix) *Ceric sulphate* 0·1*N* *in sulphuric acid* 3·5*N*

Add 5 g of ceric sulphate (see iv) to 35 ml of 10*N* H_2SO_4 and warm for 10 minutes to dissolve. Dilute to 100 ml with redistilled water and filter through a Whatman No. 42 paper. Standardize by addition of 0·5 ml of KI solution (≡0·5 mg iodine) to 10 ml of solution and titrate at 40°C with 0·15*N* arsenious acid to colourless endpoint. Adjust to 0·1*N* with 3·5*N* H_2SO_4.

(x) *Standard iodide solutions*

A. Dissolve 6·541 g of KI A.R. in redistilled water and make up to 500 ml.
(1 ml = 10 mg iodine)

B. Dilute 5 ml of A to 50 ml with redistilled water.
(1 ml = 1 mg iodine)

C. Dilute 5 ml of B to 50 ml with redistilled water.
(1 ml = 100 μg iodine)

D. Dilute 5 ml of C to 50 ml with redistilled water.
(1 ml = 10 μg iodine)

E. Dilute 5 ml of D to 50 ml with redistilled water.
(1 ml = 1 μg iodine)

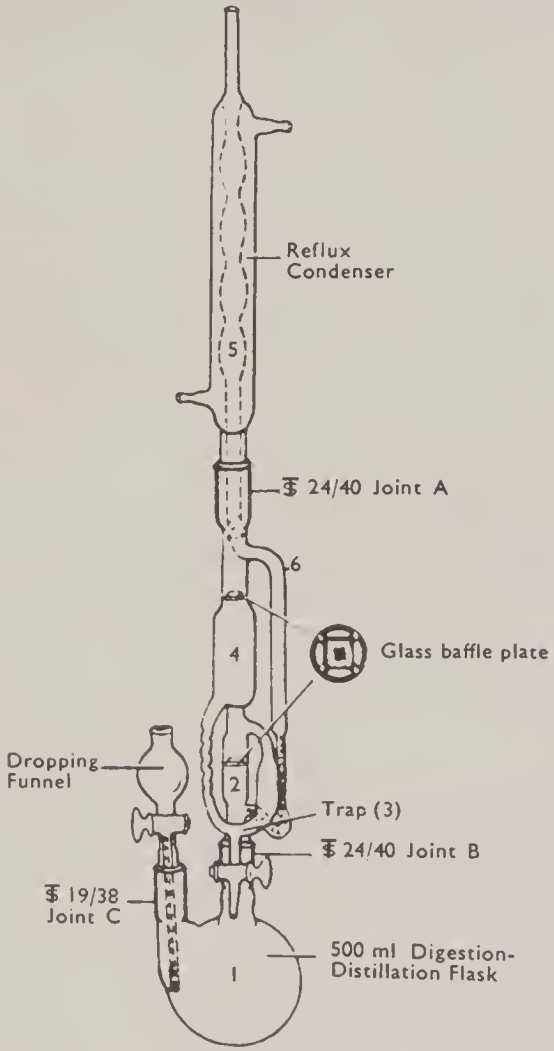
F. Dilute 5 ml of E to 50 ml with redistilled water.
(1 ml = 0·1 μg iodine)

G. Dilute 5 ml of F to 50 ml with redistilled water.
(1 ml = 0·01 μg iodine)

A, B and C: prepare every four weeks.
D and E: prepare every week.
F and G: prepare immediately before use.

Riggs' all-glass distillation apparatus

This is shown in the accompanying diagram.



The two-necked flask (1) is used both for digestion and for distillation of iodine. During the distillation procedure the digested solution in flask (1) is heated. Steam is generated which passes up column (2) to the right and downwards through the NaOH in the trap (3). The steam then courses to the left and upwards to chamber (4). From there it passes

into the condenser (5). The water of condensation drips down and is returned by tube (6) to column (2) whence it drops back into the distillation flask. Thus the apparatus permits reflux distillation of the iodine into the trap (3).

The dropping funnel inserted in the side of flask (1) is used for introduction of oxalic acid solution.

Pulfrich spectrophotometer

PREPARATION OF SAMPLES

Blood

Allow free-flowing blood to run into a clean bottle containing recrystallized potassium oxalate (2 mg/1 ml blood) as an anticoagulant.

Serum

Allow untreated blood to clot and then centrifuge for 15 minutes. Decant the serum.

Plasma protein (for protein-bound iodine determination)

Oxalate blood as described above. Centrifuge the blood at 3,500 r.p.m. for 15 minutes. Pipette off the plasma into a clean bottle (for storage keep in a refrigerator preferably at $-10°C$).

Transfer 3 ml of the plasma into a 50 ml centrifuge tube together with 10 ml of distilled water and 10 ml of 2 per cent zinc sulphate ($ZnSO_4.7H_2O$) solution. Mix with a glass rod and add 10 ml of 0·1*N* NaOH solution.

Mix again and make up to 40 ml with distilled water, removing the washed rod. Allow to stand 20 minutes to coagulate completely as indicated by a clear zone apparent at the top of the liquid. Centrifuge at 3,500 r.p.m. for 5 minutes.

Decant the supernatant liquid and discard. Add about 5 ml of distilled water to the precipitate, breaking up the latter with a glass rod. Wash down the rod with distilled water and remove.

Make up to 40 ml with a jet of distilled water and centrifuge at 3,500 r.p.m. for 4 minutes. Decant supernatant liquid and discard.

(For normal samples two washings suffice to remove " free " iodine. Samples from iodine-treated subjects require four washings.)

For the iodine determination transfer the precipitate quantitatively to the combustion flask, using 80 ml of 18*N* H_2SO_4 and a glass rod. The normal combustion and distillation is then carried out.

Milk

Preserve by addition of 1 ml of 20 per cent NaOH solution per 100 ml of milk. Store in refrigerator.

Thyroid tissue

For every gramme of tissue add 6 ml of 20 per cent NaOH solution and bring to the boil, macerating with a glass rod until the solution is homogeneous. Dilute so that 300 ml = 1 g of thyroid in 0·4 per cent NaOH.

Water

Allow water to flow into a clean glass-stoppered bottle. (Store in refrigerator.)

To the combustion flask add glass beads, copper metal, ceric sulphate and 50 ml of the water sample. Slowly add 40 ml of concentrated H_2SO_4, 5 ml at a time, while cooling in running water.

Carry out the normal procedure for combustion and distillation.

PROCEDURE

Digestion. To the combustion flask add about 20 small glass beads, 80 ml of 18*N* H_2SO_4, a piece of copper metal, 10 mg of ceric sulphate and 4 ml of the sample (*i.e.* for blood, serum, milk, and thyroid tissue—for plasma protein

or water see above). Mix until homogeneous and then add 4–5 g of potassium permanganate. Continue mixing with frequent agitation until the initial vigorous reaction subsides.

Place a thermometer (0°–200°C) in the side neck of the flask and heat very gently in a fume chamber to about 80°C, care being taken to avoid excessive foaming. When the solution is golden brown in colour increase the flame and allow the temperature to rise to 195°–200°C. When this temperature is reached allow the solution to cool to below 100°C.

Add exactly 25 ml of redistilled water (the volume of water is important for maintaining the correct distillation temperature), rinsing the thermometer (and removing it) and the necks of the flask. Mix by swirling contents. Insert a 5-inch thermometer (100°–200°C) in the central neck of the flask.

Distillation. Connect the flask to the distillation apparatus. Attach the tap funnel, filled with saturated oxalic acid solution, to the side neck of the flask and commence heating, ensuring that the condenser delivery spout is directed into the trap section. While heating up, gently rock the distillation apparatus to prevent settling of manganese dioxide.

When the temperature reaches 145°C commence to add oxalic acid solution dropwise and continue until sufficient condensate has accumulated to block the lower portion of the trap. Run 2 ml of 1 per cent NaOH down the condenser into the trap. Allow condensation to continue into the trap so that the last traces of NaOH may be washed out of the condenser. When this has been accomplished turn the condenser round so that the delivery of liquid is into the half cup leading to the reflux connection back into the flask.

Maintaining the distillation temperature at 140°–145°C,

continue addition of oxalic acid solution until the permanganate is completely reduced (solution colourless), then add 30 drops more. Continue reflux distillation for a further five minutes at 140°–145°C.

Remove the flame and run off the trap contents into a calibrated vessel. Turn the condenser so that delivery is into the trap section and wash out with two 1 ml quantities of redistilled water, adding the washings to the distillate. Make up the distillate to a suitable volume with redistilled water and mix.

Detach the distillation flask and wash out while still hot to avoid solidification of contents.

Colorimetry. Into seven test tubes pipette 0·4 ml of arsenious acid reagent. Transfer 5 ml of the distillate into one of these and pipette 0, 1, 2, 3, 4 and 5 ml quantities of the dilute iodine standard G into the remaining six. Adjust the volumes in the standard tubes to that in the unknown tube by addition of redistilled water.

Place all the tubes in a water bath at 30°C and at one-minute intervals, timed by a stop watch, add 0·3 ml of ceric sulphate reagent and mix by inversion. After 15 minutes from the time of addition of ceric sulphate remove each tube and read the colour density in the spectrophotometer against a compensating water blank using 1 cm stratum depth and a violet colour filter.

Prepare a curve from the series of standards, and read off the corresponding iodine values for the unknown. (It may be found necessary to dilute the unknown before the colorimetry is undertaken in order to get a point on the prepared curve.)

Reagent blank

The "blank value" due to iodine in the reagents is determined by carrying through the combustion and distillation procedure as for the unknown.

PLANT MATERIALS

Procedure taken from " Chemical methods for the minor elements in plants and soils." *Soils Department, New Jersey Agricultural Experiment Station*, 1952.

SUMMARY

The oven-dried sample is digested with a mixture of chromium trioxide and concentrated H_2SO_4. Iodine compounds, oxidized to iodate by this process, are reduced with phosphorous acid, and molecular iodine is distilled into an alkaline absorption solution. The iodine is reconverted to iodate by potassium permanganate oxidation. After addition of KI and release of free iodine, a starch–iodine chromogen is developed in an ice-bath. The per cent light transmission of the solution is then determined with a spectrophotometer, and the iodine concentration calculated from a previously-prepared standard curve.

For samples which have a high iodine content titration with sodium thiosulphate replaces the colorimetric procedure.

REAGENTS

(i) *Concentrated sulphuric acid*

(ii) *Chromium trioxide solution*
1000 g of chromium trioxide are dissolved in 1000 ml of distilled water. If this amount of water does not completely dissolve the salt, after standing overnight, a small additional quantity may be used.

(iii) *Phosphorous acid* 30 *per cent*

(iv) *Sodium hydroxide* 0·2*N*

(v) *Potassium permanganate* 0·2*M*
Keep in a brown bottle.

(vi) *Phosphoric acid 28 per cent*
100 ml of 85 per cent phosphoric acid diluted to 300 ml with distilled water.

(vii) *Sodium nitrite 1·5M*

(viii) *Urea 5M*

(ix) *Potassium iodide 5 per cent*
Keep in brown bottle and store in refrigerator. Make fresh twice weekly.

(x) *Sodium thiosulphate 0·01N and 0·001N*
Made by proper dilution from 0·1*N* sodium thiosulphate. About 0·2 g of Na_2CO_3 should be added to preserve the 0·1*N* sodium thiosulphate. In the refrigerator it will keep for at least two weeks.

(xi) *Potato starch solution*
1·25 g of potato starch are ground to a thin paste with a little cool water and poured slowly into 500 ml of boiling water. Boil for 15 minutes and add 0·5 g of salicylic acid as a preservative. Cool and filter. Renew every 3–4 days. Keep in a refrigerator.

(xii) *Standard iodine solution*
A stock standard is prepared by dissolving 0·1686 g of reagent grade KIO_3, which has been dried at 120°C for 24 hours, in 1 litre of distilled water. This solution contains 100 μg of iodine per ml. Appropriate dilutions of the stock standard are made as desired.

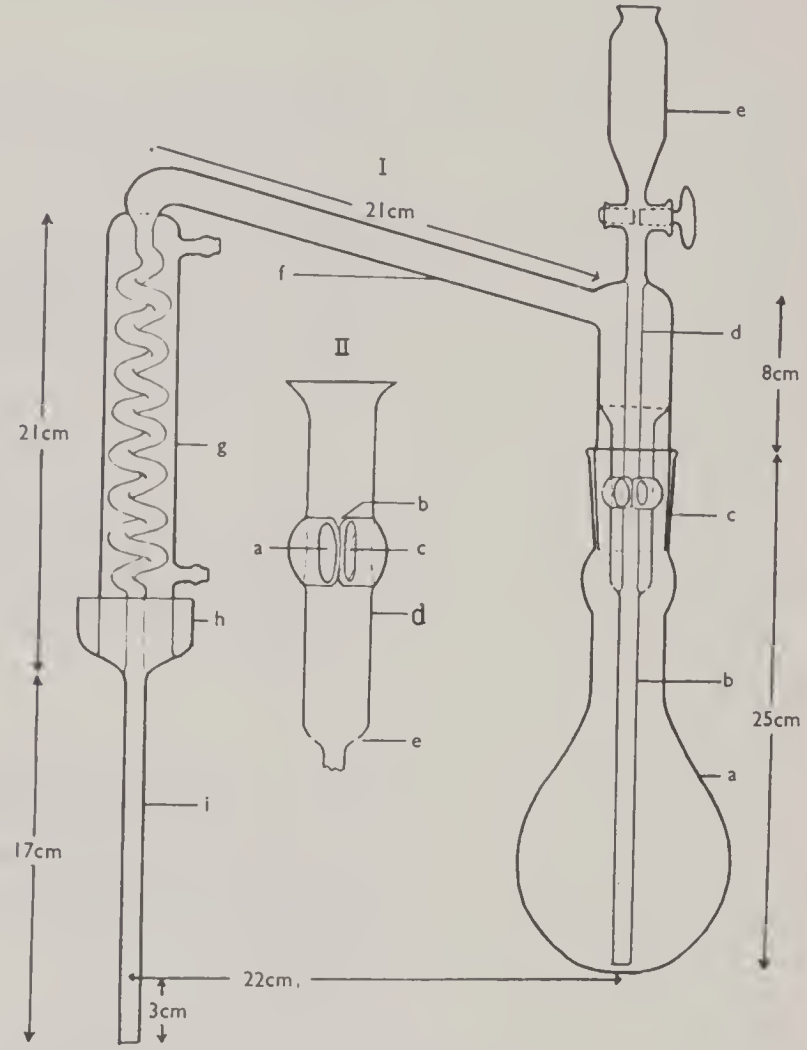
SPECIAL APPARATUS

Micro-iodine distillation apparatus

This is shown in the diagram overleaf.

The digestion and distillation flask (I-a) may be of any size from 300 to 1200 ml. (I-c) is a standard joint of 30 mm. The coil condenser (I-g) has a dew-collecting cup (I-h)

attached. Tubes having the following respective inside and outside diameters are recommended for the various pieces: (I-b) 3 and 9 mm; (I-d) 3 and 6 mm; (I-f) 11 and 15 mm; and (II-d) 13 and 15 mm.



The apparatus may be mounted on one stand. It is washed by drawing water through the condenser into a flask with a stopper fitted for the joint.

(II) presents the entrainment trap, designed for this distillation. Its efficiency has been tested and has been found to compare well with that of other entrainment traps with

larger or more intricate construction. Vapours on leaving the flask enter at (II-a), follow the concentric tube around tube (II-d) and enter tube (II-d) through opening (II-c). The concentric tube is closed off at (II-b), between (II-a) and (II-c). Three small holes, 0·2 mm in diameter, are located at (II-e), the bottom of (II-d), in order to permit condensed liquid to flow back into the flask. The concentric tube should have an inside cross-section area of approximately 30 sq mm.

Four units can be operated at the same time by connecting the cooling jackets of the condensers.

Beckman spectrophotometer

With optically-matched Corex glass cells having a 1 cm light path.

PROCEDURE

Digestion. One g of oven-dried material is weighed and transferred to the distillation flask. If the flask has been used recently and is still moist, a paper funnel may be made and inserted in the neck of the flask.

Exactly 10 ml of chromium trioxide solution are added from a volumetric pipette, and mixed with the sample. Fifty ml of concentrated H_2SO_4 are then run into the flask from a large burette under a hood. A small amount of acid should be run in at first. When the initial reaction has subsided the remainder may be added. Some of the acid should wash the neck and sides of the flask to remove adhering particles of plant material.

A thermometer held by a rubber stopper is inserted in the flask. The stopper should have a second, rather large, hole to permit gases to escape.

The flask is heated to 200°–220°C and held at this temperature for about 5 minutes. In the case of a blank it may be necessary to continue heating slightly longer than 5

minutes. During the heating the flask is swirled constantly to prevent the formation of any precipitate. The reaction is complete when the production of bubbles by the liquid is markedly reduced and the solution changes from a dirty brownish-green colour to a dark brilliant green.

The solution is permitted to cool down to approximately 70°C and 100 ml of distilled water are added slowly, the sides of the flask and the thermometer being washed down carefully in the process. Four to 6 glass beads are added to prevent bumping and the contents of the flask are mixed well by swirling the flask.

Distillation. The flask is connected to the distillation apparatus. The end of the condenser is submerged in 1 ml of 0·2*N* NaOH plus 5 ml of distilled.water in a 100 ml beaker.

The solution is heated gently until boiling occurs, when the temperature may be raised. After the distillate begins to come over, 4 ml of 30 per cent phosphorous acid are added through the delivery funnel. The phosphorous acid may be added all at once, but the stopcock should be closed immediately after the solution has passed through it. After the addition of phosphorous acid the solution may boil rather violently for a few seconds and it is often advisable to reduce the heat during the addition, as an explosion may result otherwise.

The distillation is continued until approximately 50 ml of distillate have collected in the receiving beaker. The beaker is then lowered until the condenser stem is free of the solution (in order to prevent any sucking back of the distillate into the apparatus). The heat source is removed and the stopcock is opened carefully to relieve the pressure in the distilling flask.

After the apparatus has cooled it is disconnected and rinsed with distilled water.

Oxidation. The distillate is slowly evaporated on a hot-

plate to 5–10 ml. At this point the sample may be collected and kept for several days before the procedure is continued. Care should be taken to keep the solution free from dust and fumes.

To oxidize the sample 1 drop of 0·2*M* potassium permanganate is added to the hot solution (2 drops in the case of a sample known to have a high iodine content). Heating is continued for 5 minutes and then 3 drops (0·09 ml) of 28 per cent phosphoric acid are added with an eye dropper. The sample may change colour slightly at this point but this can be disregarded. If the sample discolours completely 2 or more drops of 0·2*M* potassium permanganate are added. Usually such a sample will turn brownish, but it will still give a satisfactory result.

After the addition of the phosphoric acid, heating is continued for 2 minutes and then 2 drops of 1·5*M* sodium nitrite are added to the solution with an eye dropper. This reduces the excess potassium permanganate and destroys the colour. The sodium nitrite should *not* run along the walls of the beaker.

The beaker is swirled at this point (the solution should be completely colourless). If any brown precipitate remains, a few more drops of sodium nitrite may be added to dissolve it. However, a result obtained with such a sample should be viewed with suspicion.

Heating is continued for 2 minutes after the addition of sodium nitrite and 5 drops of 5*M* urea are then added with an eye dropper. The sides of the beaker are washed down with distilled water and heating continued for at least 5 minutes or until the volume of the solution is reduced to approximately 2 ml. The solution is then permitted to cool to room temperature.

Development of the starch–iodine chromogen

(a) *Samples with high iodine content* (above 10 μg). The

sample is cooled in a refrigerator and 1 ml of 5 per cent KI solution is added. A sample high in iodine should turn yellow at this point. It is immediately titrated with sodium thiosulphate (either 0·01*N* or 0·001*N*, depending on the amount of iodine in the sample) until the yellow colour nearly disappears. Ten drops of starch solution are then added from an eye dropper and the titration continued until the blue starch–iodine colour vanishes.

If the sample remains colourless after the addition of KI, add 10 drops of starch solution immediately and titrate.

The end point of the titration is very definite and the solution should remain colourless. If a blue colouration reappears after standing, the sample must be run again as the result is unreliable.

1 ml of 0·01*N* sodium thiosulphate ≡ 211·6 μg of iodine
1 ml of 0·001*N* sodium thiosulphate ≡ 21·16 μg of iodine

(b) *Samples with low iodine content* (0·1–10·0 μg). The sample is transferred into a test tube marked at the 5, 10 and 15 ml levels. Solution and washings after the transfer should be less than 4·5 ml.

The test tube is placed in an ice-bath for 1 minute and then 1 drop of 5 per cent KI and 10 drops of starch solution are added with an eye dropper. The solution is mixed and the volume brought up to exactly 5 ml. The test tube is then returned to the ice-bath until needed.

To determine the light transmission the solution is transferred from the test tube into a corex spectrophotometer cell having a 1 cm light path. The per cent transmission is read with a Beckman spectrophotometer using a wavelength of 575 mμ and a slit width of 0·05 mm.

Should the transmission reading be below 20 per cent, the solution can be returned to the test tube and be diluted to either 10 or 15 ml. The percentage transmission is then read at these dilutions.

The amount of iodine present in the sample is obtained from a curve prepared from appropriate dilutions of the standard iodine solution.

Standard curves

Houston (1950) obtained a straight curve for 0·1 to 5·0 μg of iodine when plotted on semi-log paper. With the spectrophotometer in use at the New Jersey Agricultural Experiment Station this was not possible. However, for 5–10 μg at higher dilutions straight curves were obtained when plotted on semi-log paper. The curves obtained were as follows:

μg of iodine in 5 ml of water	*% transmission at 575 mμ*
0·10	99
0·25	97
0·50	89
1·00	78
2·50	45
5·00	18
μg of iodine in 10 ml of water	
4	56
6	46
8	36
10	26
μg of iodine in 15 ml of water	
4	75
6	62
8	54
10	45

Reagent blank

A blank determination must be run for each new batch of chromium trioxide, which usually contains some iodine

(the reagents required for a 1 g sample showed a blank varying from 0·2 to 0·4 μg of iodine).

Larger samples

For most analyses at the New Jersey Agricultural Experiment Station a 1 g sample in a 300 ml distillation flask was used. A 2 g sample in a 600 ml flask may be used for material having an iodine content below 100 μg/kg. For a 2 g sample the following reagents are doubled in quantity:

Chromium trioxide
Concentrated H_2SO_4
Phosphorous acid
Distilled water

SOILS

Procedure taken from " Official methods of analysis of the Association of Official Agricultural Chemists." Washington, D.C.: *A.O.A.C.*, 1955, 8th Edition.

SUMMARY

Organic material in the soil is oxidized by an alkaline fusion technique. Iodine is isolated by alcoholic extraction and determined, in a carbon bisulphide solution, by colorimetric comparison with a known standard.

REAGENTS

(i) *Potassium hydroxide pellets, iodine-free*

(ii) *Potassium bisulphite*

(iii) *Hydrochloric acid*, 1+1
One volume concentrated HCl plus 1 volume of water.

(iv) *Potassium carbonate solution, saturated*

(v) *Ethyl alcohol, 95 per cent*

(vi) *Sulphuric acid*, 1+1
One volume concentrated H_2SO_4 plus 1 volume of water.

(vii) *Carbon bisulphide*

(viii) *Potassium nitrite or sodium nitrite, iodine-free, 10 per cent*

(ix) *Distilled water*

PROCEDURE

Digestion. Place in a clean 400 ml iron crucible 5 g of air-dried soil, ground to pass a 100-mesh sieve, 10 g of iodine-free KOH pellets and 5 ml of water. Stir with a clean piece of No. 6 iron wire until most of the pellets have dissolved.

Put the crucible in a 4·5 inch bunsen tripod and heat moderately. Stir the contents rapidly until the water has been driven off and a dry granular fused mass remains. Avoid heating the crucible to redness after the water has been expelled.

Cool the crucible, add about 50 ml of water and allow to stand with occasional stirring until the fused mass has slaked to a sludge. Transfer the contents of the crucible to a 500 ml beaker, making sure that nothing remains in the crucible by rubbing and washing the inside walls thoroughly. Add a small strip of litmus paper, about 0·1 g of potassium bisulphite, and HCl (1+1), stirring until the contents of the beaker have an acid reaction and a distinct odour of SO_2 can be detected. Add saturated K_2CO_3 solution from a short-stemmed pipette, stirring until the entire mass has an alkaline reaction.

Pour the precipitate of silica, and of iron and aluminium hydroxides onto a folded filter and wash well with 25 ml portions of hot water, allowing each portion to drain completely. (The volume of filtrate and washings should be about 500 ml.) Transfer the filtrate to a porcelain dish and evaporate on a water bath until a sludge of salts remains. (A small current of compressed air directed onto the surface of the solution during heating will hasten evaporation.)

Remove the dish from the water bath and add 50 ml of alcohol to the hot sludge of salts. Stir until the dish has attained room temperature. (Salts assume a pasty consistency with much stirring while they are hot, and this condition facilitates solution of the KI in the alcohol.) Decant the alcoholic extract through a small folded filter into a beaker. Further extract the residue with one 25 ml and one 10 ml portion of alcohol and decant through the filter into the beaker containing the first alcoholic extract.

Dissolve the salts that adhere to the filter paper and those in the dish with hot water and evaporate to a sludge. Extract the sludge with one 25 ml and two 10 ml portions of alcohol, as directed in the treatment of the first sludge of salts, and repeat the process of extraction using three 10 ml portions of alcohol.

Combine the alcoholic extracts and evaporate to dryness. Dissolve the residue in a small quantity of hot water, rinse into a 250 ml beaker, and evaporate to dryness. Dissolve the residue, in a few drops of hot water and a drop of saturated K_2CO_3 solution, and add 10 ml of alcohol. Stir the precipitated salts rapidly with a glass rod for about 10 minutes and decant the alcoholic extract through a small filter into a 150 ml beaker. Stir the residue, extract with two 5 ml portions of alcohol and decant through the filter into the beaker, rinsing the filter with a few ml of alcohol. Evaporate the extract to dryness.

Dissolve the residue, consisting of about 0·05 g, in a few

drops of hot water, rinse into a 25 ml platinum or sillimanite dish, evaporate to dryness, and dry at 100°C for 1 hour. Heat the dish at about 400°C in an electric furnace having a pyrometer attachment until the organic matter is burned or charred. After cooling the dish, dissolve the residue in a few drops of water at room temperature, filter the solution, and wash into a 30 ml separator. (The solution should be colourless, slightly alkaline, and have a volume of about 5 ml. If the solution is tinted, evaporate to dryness and burn again.)

Acidify slightly with H_2SO_4 (1 + 1), add about 0·01 g of pure potassium bisulphite to the separator, stopper, and shake for a few seconds to reduce any iodate to iodide. Remove the stopper and add 1 ml of pure carbon bisulphide and about 2 ml of the 10 per cent solution of pure, iodine-free, potassium nitrite or sodium nitrite. Stopper, and shake the separator vigorously for about a minute.

Place the separator in a stand and allow the carbon bisulphide containing the iodine to collect and settle for about 5 minutes. If the carbon bisulphide is light pink in colour, it contains all the iodine; if it is deep pink, run it carefully into a centrifuge tube and further extract the solution in the separator with 1 ml portions of carbon bisulphide until the last portion is only slightly pink. Combine the carbon bisulphide extracts and centrifuge.

Iodine estimation. Place a portion of clear extract in the micro-cup of a colorimeter and compare quickly with a freshly prepared iodine standard having comparable depth of colour.

IODIZED SALT

Procedure taken from " Official methods of analysis of the Association of Official Agricultural Chemists." Washington, D.C.: *A.O.A.C.*, 1955, 8th Edition. This is similar to the method recommended by the W.H.O. Study-Group on Endemic Goitre, London, December 1952.

SUMMARY

The iodide in the salt (iodine being present as NaI or KI) is oxidized to iodate by bromine. If iodine is present as iodate the oxidation step is omitted. After excess bromine has been removed KI is added. This releases free iodine which is then titrated with sodium thiosulphate using starch as indicator.

REAGENTS

(i) *Methyl orange indicator*

(ii) *Sulphuric acid* 2*N*

(iii) *Sodium thiosulphate* 0·005*N*
Recently standardized.

(iv) *Phenol* 5 *per cent*

(v) *Potassium iodide* 10 *per cent*

(vi) *Starch* 1 *per cent*
Freshly prepared.

(vii) *Sodium chloride A.R.* 20 *per cent*

(viii) *Bromine water*
Prepare a strong solution from bromine A.R. Determine the approximate concentration (mg bromine/ml) by adding a measured volume (3–5 ml) from a burette to a flask containing 50 ml of water, 5 ml of 10 per

cent KI and 5 ml of " 1+9 " H_2SO_4 (1 volume of concentrated H_2SO_4 plus 9 volumes of water). Titrate the liberated iodine with 0·1*N* sodium thiosulphate.

(ix) *Potassium iodide control solution*
Dissolve 0·328 g of dried KI A.R. in water and make up to 250 ml. Dilute 50 ml to 250 ml and use 5 ml of this final solution for control (5 ml ≡ 1·0 mg iodine ≡ 1·312 mg KI).

(x) *Distilled water*

PROCEDURE

Dissolve 50 g of the salt sample in water and make up to 250 ml in a volumetric flask.

Oxidation. Pipette 50 ml of the solution into a 200 ml Erlenmeyer flask, and neutralize to methyl orange with 2*N* H_2SO_4. Add bromine water dropwise from a burette in quantity equivalent to 20 mg of bromine. After a few minutes destroy the greater portion of the remaining free bromine by adding 1 per cent sodium sulphite solution dropwise while mixing. Wash down the neck and sides of the flask with water and complete the removal of the free bromine by addition of 1 or 2 drops of 5 per cent phenol solution.

Iodine estimation. Add 1 ml of 2*N* H_2SO_4 and 5 ml of 10 per cent KI solution. Titrate the liberated iodine with 0·005*N* sodium thiosulphate solution adding 1 ml of starch indicator near the end of the titration (1 ml 0·005*N* sodium thiosulphate ≡ 0·1058 mg iodine ≡ 0·1388 mg KI).

Note

The determination should be corrected by a blank test of the reagents. One or more control determinations should

be made for comparison using 50 ml of 20 per cent NaCl solution to which has been added appropriate quantities of the control solution of KI.

MODIFICATION OF THE PROCEDURE

Some slight modifications of the procedure have been introduced by the Institute of Nutrition of Central America and Panama (INCAP), Guatemala, in order to improve reproducibility.

Addition of bromine

Add bromine water dropwise from a burette in quantity equivalent to 10 mg of bromine *and let stand for* 10 *minutes.*

Destruction of excess bromine

Add dropwise from a burette 1 per cent sodium sulphite solution while whirling the flask, *until only a pale yellow colour remains.* Then add 2 drops of 5 per cent phenol solution.

Liberation of iodine for titration

Add 1 ml of 2*N* H_2SO_4, 0·5 g of KI and *let stand in the dark for* 10 *minutes.* Then titrate with sodium thiosulphate as indicated.

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Organic material in the sample is destroyed by alkaline incineration in a nickel dish. Iodine is extracted from the ash with alcohol and oxidized to iodate with bromine water. Addition of potassium iodide liberates free iodine and this is titrated with sodium thiosulphate using starch as indicator.

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The sample is treated with a dichromate–sulphuric acid mixture in the presence of small amounts of cerous sulphate to oxidize the iodine to iodic acid. This is reduced by phosphorous acid and the free iodine is steam-distilled into potassium carbonate solution under reduced pressure. The iodide is then oxidized with bromine, and the iodine is liberated with potassium iodide and titrated with sodium thiosulphate using starch as indicator.

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The general procedure is described for the determination of micro-quantities of iodine by means of the catalytic effect of iodide on the reduction of yellow ceric sulphate to colourless cerous sulphate by arsenious acid.

1938. ELMER, A. W. Iodine metabolism and thyroid function. London: *Oxford University Press*, 1938.

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Organic material is digested in a chromic acid–sulphuric acid solution. Iodine, oxidized to iodate by this process, is reduced by phosphorous acid to molecular iodine which is isolated by simple distillation into an alkaline absorption solution. Potassium permanganate is added to oxidize the iodine to iodate. Free iodine is liberated by potassium iodide and titrated with sodium thiosulphate using starch as indicator.

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Organic matter is destroyed by dry ashing in a muffle furnace. Iodine is extracted from the ash with dilute sulphuric acid and determined colorimetrically by means of its catalytic action on the reduction of ceric sulphate.

1944. Talbot, N. B., A. M. Butler, A. H. Saltzman and P. M. Rodriguez. The colorimetric estimation of protein-bound serum iodine. *Journal of Biological Chemistry*, 1944, v. 153, pp. 479–488.

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Method for estimation of iodine in rocks, soils, waters, and plant and animal tissues. Procedure is similar to that described by Matthews *et al.* (1938) but with a colorimetric finish based on the development of a starch–iodine chromogen.

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1949. Shahrokh, B. K., and R. M. Chesbro. Microdetermination of iodine in materials with high organic content. *Analytical Chemistry*, 1949, v. 21, pp. 1003–1005.

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1950. Chaney, A. L. Instrumental improvements for microdetermination of protein-bound iodine in blood. *Analytical Chemistry*, 1950, v. 22, pp. 939–942.

A photoelectric recording colorimeter, with a very stable light source, is described. The colorimeter is specifically designed for measurement of trace quantities of iodine.

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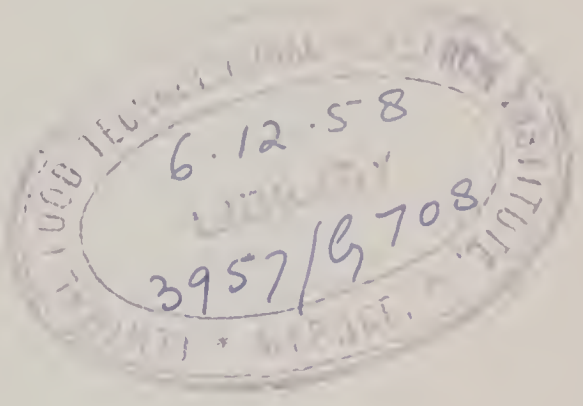

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